

International Application Number: PCT/EP98/04816

Applicant: KOSZINOWSKI, Ulrich H. et al.

Representative's file number: PCT897-03166/VL

Date: November 4, 1999

New Patent Claims

1. Recombinant vector containing infectious viral genome sequences having a size larger than 100 kb, as well as sequences of a cloning vehicle which are capable of DNA replication in a host cell, with the cloning vehicle being a bacterial artificial chromosome (BAC).
2. Recombinant vector according to claim 1, characterized in that the infectious viral genome sequences have a size larger than 200 kb.
3. Recombinant vector according to claim 1 or 2, characterized in that the viral genome sequences derive from a DNA virus.
4. Recombinant vector according to claim 3, characterized in that the viral genome sequences derive from a herpesvirus.
5. Recombinant vector according to claim 4, characterized in that the viral genome sequences derive from a betaherpesvirus.
6. Recombinant vector according to claim 5, characterized in that the viral genome sequences derive from human cytomegalovirus.

7. Recombinant vector according to claim 5, characterized in that the viral genome sequences derive from mouse cytomegalovirus.
8. Recombinant vector according to claim 4, characterized in that the viral genome sequences derive from a gammaherpesvirus.
9. Recombinant vector according to claim 4, characterized in that the viral genome sequences derive from murine gammaherpesvirus 68 (MHV 68).
10. Recombinant vector according to one or several of the preceding claims, characterized in that the sequences of the cloning vehicle are flanked by identical sequence sections.
11. Recombinant vector according to one or several of the preceding claims, characterized in that the sequences of the cloning vehicle are flanked by recognition sequences for sequence-specific recombinases and/or by restriction sites which do not appear in the rest of the vector.
12. Recombinant vector according to claim 11, characterized in that the recognition sequences for sequence-specific recombinases are loxP sites.
13. Recombinant vector according to one or several of the preceding claims, characterized in that selection and/or marker genes are additionally contained.
14. Cell containing a recombinant vector according to any one of claims 1 to 13.
15. Method for producing a recombinant vector according to one or several of claims 1 to 13, comprising the following steps:
- a) introducing a sequence (1), containing sequences of a cloning vehicle which

are capable of DNA replication in a host cell, with the cloning vehicle being a bacterial artificial chromosome (BAC), into a cell containing infectious viral genome sequences, and

- b) recombining sequence (1) of step a) with the viral genome sequences to obtain a recombinant vector.

16. Method according to claim 15, characterized in that step b) is carried out via homologous recombination.

17. Method according to claim 15 or 16, characterized in that a eukaryotic cell is used as the cell in step a).

18. Method according to claim 17, characterized in that the eukaryotic cells are mammalian cells.

19. Method according to claim 18, characterized in that the eukaryotic cells are primary fibroblasts, human foreskin fibroblasts (HFF) or mouse embryonic fibroblasts.

20. Method according to claim 19, characterized in that the eukaryotic cells are NIH3T3 fibroblasts.

21. Method according to any one of claims 15 to 20, characterized in that sequence (1) is introduced into the cells by a calcium phosphate precipitation method, a lipofection method or an electroporation method.

22. Method according to any one of claims 15 to 20, characterized in that sequence (1) is introduced into the cells by a further viral vector.

23. Method according to claim 15, characterized in that a bacterial organism is used as the cell in step a).

24. Method according to claim 23, characterized in that *E. coli* is used as the cell in step a).

25. Use of a recombinant vector according to one or several of claims 1 to 13 for the mutagenesis of the infectious viral genome sequences contained therein.

26. Method for the mutagenesis of viral genome sequences contained in a recombinant vector according to one or several of claims 1 to 13, the method comprising the following steps:

A) introducing the recombinant vector into a bacterial host cell; and

B) mutagenizing the viral genome sequences.

27. Method according to claim 26, characterized in that the mutagenesis of viral genome sequences is carried out by homologous recombination of the recombinant vector with DNA molecules contained in the bacterial host cell.

28. Method according to claim 27, characterized in that the homologous recombination of the recombinant vector is carried out with a mutant allele.

29. Method according to claim 26, characterized in that the mutagenesis of the viral genome sequences is carried out with the help of a transposon.

30. Method according to one or several of claims 26 to 29, characterized in that the recombinant vector is obtainable by a method according to one or several of claims 15 to 24.

- odd
a'